Remarks

Election/Restrictions

The Applicant affirms the election without traverse of Claims 1-22 and 40 on 12/8/05. Thus, Claims 23-29 are withdrawn and Applicant has, accordingly, cancelled Claims 23-29 as noted in the Listing of Claims, pages 4-12 of this paper.

Amendments to the Specification

Beginning on page 2 of this paper the Applicant requests several amendments to the specification.

First, the Applicant wishes to claim priority benefit from the provisional filing of March 14, 2003.

The 6 additional amendments to the specification, of paragraphs [0026], [0030], [0034], [0039], [0043] and the top column heading line of Table I, are requested to correct inadvertent omissions of words (paragraphs [0030] and [0034]), to enter clarifying wording (paragraphs [0026], [0039] and [0043]) and to correct the inadvertent incorrect notation for di-sodium phosphate (originally noted as Na_2PO_4 rather than Na_2HPO_4).

None of the amendments to the specification contain new matter.

Amendments to the Claims

As noted above, claims 1-22 and 40 are pending in the present application. The Applicant, in the Listing of Claims, herein requests amendment of claims 1, 2, 3, 7, 8, 10 and 40 in response to the Examiner's remarks and rejections. The Applicant additionally requests the Examiner's consideration of newly added claims 41 – 53. The requested

amendments of the originally filed claims and the newly added claims contain no new matter.

Remarks in response to the rejections of Claims 1-22 and 40

The Applicant has made amendments to the Claims solely to advance his business interests and reserves the right to prosecute claims that are the same or similar to those amended herein in the future. None of the amendments to the pending claims is intended to narrow the scope of any of the amended claims within the meaning of *Festo* [Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., 344 F3d 1359, 68 USPQ 2d 1321 (Fed. Cir. 2003)] or *Honeywell* [Honeywell Interm. Inc. v. Hamilton Sundstrand Corp., 370 F.3d 71 SPQ 2d 1065 (Fed. Cir. 2004)].

Rejection under 35 USC § 112, first paragraph:

Claims 1-22 and 40 have been rejected under 35 USC § 112, first paragraph as failing to comply with the written description requirement. Specifically, the Examiner states that:

> "Claims 1 – 22 and 40 are genus claims in terms of a method using any bacteria, any promoter, using any inducer and any metabolite which prevents induction by said inducer, in any culture medium, and in terms of methods in which one component (*i.e.*, promoter, inducer, metabolite, culture medium or component thereof) is specified with other components broadly claimed."

and,

> "While the specification provides general information on autoinduction of the expression of a protein of interest in bacterial cells by using appropriately selected promoter systems, culture components, inducers and metabolites which will prevent induction until a later stage of growth is achieved, the provision of guidance for one specific example of such a

> system, does not provide an adequate description of the entire genus of the claimed methods."

In response, the Applicant's request the Examiner's consideration of the following remarks and discussions.

First, it is the Applicant's understanding that U.S. patent practice provides that an application need not provide every embodiment of an invention for a genus Claim to issue.

> ... applicant need not describe all actual embodiments. [MPEP 2164.02].

and,

> For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation. [MPEP 2164.02].

In this vein, the Applicant respectfully submits that the "genus" is not as broad as it may appear, particularly in light of the amendment of Claim 1 that is currently requested.

Claim 1 is not drawn to "any bacterial cell". Rather, Claim 1, as originally filed and as amended herein for clarification purposes, is drawn to bacterial cells adapted for use in expression of cloned DNA. Claim 1 is not drawn to "any promoter", but rather to a promoter inducible by an exogenous inducer. Those of ordinary skill in the art of expression of cloned DNA in bacterial cells, which cells are adapted for such purposes, are familiar with a variety of promoters that

are used for such controllable expression. Further, Claim 1 is not drawn to "any inducer", but rather to inducers that are routinely used in such inducible expression systems. Inducible operons and the promoters of such operons and the inducers of such operons are well known in the art, and the inducers for example can comprise lactose, galactose, arabinose, and etc. Nor is Claim 1 drawn to "any metabolite which prevents induction by said inducer, in any culture medium." Rather, Claim 1 is drawn to a metabolite that can prevent induction of a promoter by virtue of its being preferentially metabolized by the bacterial cells. Thus, in the "early stages of growth" (i.e., in early log phase – see paragraphs [0035], [0039] and [0041]) in which cells are rapidly dividing, the metabolite excludes uptake of the inducer by the cells and thus precludes induction by the inducer. However, in the "later stages of growth" (i.e., after mid-log and into late log phase), in which cell division rates are slowing, the metabolic state of the cells and the concentration of the metabolite dictate that the cells recognize and take up the inducer, which in turn permits induction of the inducible promoter. Furthermore, the invention is limited to a medium wherein the medium is capable of supporting growth of the bacteria to a density where the blocking metabolite(s) can be/has been metabolized. In fact the specification provides detailed descriptions of various media in which this can occur, including rich media, fully defined minimal salt solution media, media containing and not containing glucose, media containing glucose and additional sources of carbon (e.g., glycerol, succinate, malate, etc.), media containing a single amino acid and media

containing mixtures of amino acids. In each case one or more metabolite(s) in the medium causes the cells to use a preferred carbon source(s) for initial growth (early stage of growth/early log phase) and, once that carbon source is sufficiently depleted through cell growth (i.e., at a later stage of growth/late log phase), the inducer is taken up by the cells and induction proceeds "automatically".

The present application provides working examples teaching the auto-induction system of the present invention. For examples, see, paragraphs [0035], [0036], [0043] and [0048] and the information provided in Tables 1 and 2. In this regard, the Applicant asserts that the specification as filed provides sufficient written description for one skilled in the art to practice the present invention in systems utilizing other bacteria, promoters, culture media, inducers and metabolites which prevent induction by said inducer. "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." [*In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983)]. Because the experimentation necessary to extend the present invention to other systems is not complex but merely iterative and is routinely practiced in the art, one of ordinary skill in the art would readily adapt the teachings of the present invention to expression systems that have been developed which utilize other bacterial species. Thus issuance of a patent should not be precluded on grounds of "undue" experimentation. As an example, the auto-induction method of the present invention has been easily extended to a promoter inducible by arabinose (see Studier, F. W. (2005) "Protein production by auto-induction in high-density shaking cultures", Protein Expression and Purification 41: 207-234),

thereby showing that the present specification is in compliance with the first paragraph of 35 U.S.C. 112 in regards to Claim 1 as amended.

In view of the foregoing comments, the Applicant respectfully requests that the rejection under 35 USC §112 paragraph 1 be withdrawn.

Rejection under 35 USC § 112, second paragraph:

Claims 1-22 and 40 have been additionally rejected under 35 USC §112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner notes:

> "Claim 1, and by dependence, claims 2-22 and 40, are vague and indefinite in the recitation of the phrases "in the early stages of growth" and "at a later stage of growth". These phrases are relative and it cannot be clearly determined what is intended. Therefore the metes and bounds of the intended subject matter cannot be determined."

While the Applicant respectfully disagrees, to advance this case to readiness for allowance, Claim 1 has been amended to remove any question as to whether the metes and bounds of the intended subject matter can or cannot be determined.

In support of the Applicant's differing viewpoint and in support of the clarifying amendment of Claim 1, particularly with respect to paragraph (a) ii, it is well known by experts as well as by those having only very basic knowledge of bacterial cell batchwise growth that upon inoculation of a medium with a bacterial inoculum, there will be a lag phase (very early growth) in which the cells adapt to the resources provided in the medium, which is followed by a logarithmic increase in cell number (log phase). The log

phase ends as cell division slows and eventually the increase in cell number ceases at saturation/stationary phase. Log phase is characteristically considered to be comprised of two (2) stages – early stage and later stage. In the early stage the cells begin the doubling process and increase exponentially with a doubling time related to the resources provided in the medium. As the nutrient resources in the medium are depleted by the increasing numbers of cells, the metabolic state of the cells adjusts and the rate at which the cells grow and divide begins to slow. This is identifiable as the inflection point in the growth curve (mid-log phase). Following mid-log, the doubling time of the cells increases (i.e., it takes longer and longer for a cell to divide) as the cells, having depleted the medium of one or more preferred nutrients, adjust to alternate resources in the medium. This is generally considered by those practiced in the art of growing and maintaining bacterial cultures as the "later stages" of growth, or late log phase. It is generally known that two to four cell divisions may occur after the inflection point (i.e., after mid-log). It is during this time, as the cells approach saturation, that auto-induction occurs since the metabolic state of the cells has adjusted to the remaining resources provided in the medium.

Thus the Applicant respectfully submits that Claim 1, as originally filed, and more clearly as amended herein, is well understood by those practiced in the art of growing and maintaining cells cultures and is very clearly understood by those practiced in the art of expression of cloned DNAs in bacterial cells. Therefore, the Applicant request that the rejection of the claims under 35 USC §112, second paragraph be withdrawn.

Rejection under 35 USC § 102 (b)

Claims 1-20 have been rejected under 35 USC § 102(b) as being anticipated by Hoffman et al. (1995).

In response the Applicant requests the Examiner's consideration of the following remarks in which the differences between the present invention and the teachings of Hoffman et al. are reviewed.

The primary difference between the reference cited by the Examiner and the present invention is the method in which the exogenous inducer (for example, lactose) is made available to the cells in the culture. Hoffman makes the inducing agent available by changing the media entering the fed-batch culture from a glucose-based medium to a medium containing both glucose and lactose. [see, p 647, second column, first full paragraph and Table 1]. This is a most basic difference from the system taught in the present invention wherein both inducer-excluding metabolites (carbon sources such as glucose, amino acids, glycerol, succinate, etc.) and the inducer are supplied in the culture medium at the beginning of the culture cycle. In the present invention, inducer is only accessed or taken up by the cells after the available more preferred metabolites in the culture medium are depleted by cellular metabolism and growth. In other words, the present invention teaches a culture system that is designed so that inducer is taken up by the cells only when the culture reaches a density useful for production of target protein. Unlike Hoffman, this is achieved in the present invention without any additional manipulation of the culture or intervention by the user after the initial inoculation of the culture vessel with the bacterial cells. The culture system taught by Hoffman requires

user intervention in order to induce the culture cells to produce protein. The method of the present invention is superior to the method taught by Hoffman because it eliminates considerable user intervention, and, unlike Hoffman, is readily extended to the induction of many cultures in parallel.

Another difference between the present invention and the teachings of Hoffman et al. is that Hoffman uses a fed-batch culture system. A fed-batch system provides for continuous additions of fresh media and nutrients to the culture (without removing cells or effluent). This differs from the culture system of the present invention. The culture system of the present invention is a batch system (not the fed-batch system of Hoffman). In fact, independent Claims 1, 46, 50 and 53 are limited to a "batchwise" culture system. In a batchwise system fresh media, nutrients and other agents are not added during the growth of the bacterial cells. Rather, the media is placed into the culture vessel, inoculated and bacteria are removed from it at the end of the culture run, usually for the purification of the desired protein.

One additional distinction between the teachings of the present invention and that of Hoffman et al. is that the use of a batchwise system over a fed-batch system necessitates the solving of several problems that do not need to be solved in the fed-batch process. For example, a batch system is much more susceptible to detrimental changes in pH. As stated in the present specification, "...the main limitation in achieving high cell densities appears to be maintaining the pH of the culture near neutral, since metabolism of glucose can produce substantial amounts of acid." (see paragraph [0018]). Unlike Hoffman, where non-aqueous NH_4OH is constantly metered into the culture to maintain

pH ("pH was maintained at 7.2 ± 0.1 by the controlled addition of neat NH_4OH"; see, p 649, first column, first full paragraph), the present invention maintains pH by the use of a specific selection of carbon sources. The acidification of culture medium when glucose is a main carbon source is discussed in paragraph [0016] and use of phosphate and additional carbon sources (e.g. succinate, fumarate, malate) to counteract this is discussed in paragraphs [0018], [0019], [0020] and [0022]. Counteracting the increased acidity of the medium so as to optimize cell growth is important for growing the bacterial cells under conditions for maintaining the cultures (i.e. to produce storage cell stocks) and for culturing for production of auto-induced target RNA or protein.

Consideration of newly added Claims 41-53:

In addition to the above remarks and reasoning with respect to the withdrawal of rejections of claims 1-22 and 40, the Applicant respectfully requests the Examiner's consideration of newly added claims 41 through 53. Claims 41 through 45 depend ultimately from Claim 1. As such, they are fully supported by the documentation in the specification and the Applicant submits they are allowable given their dependence from Claim 1 as amended herein. Independent Claims 46 and 50, and the claims that depend therefrom, serve to restate the present invention in a more succinct fashion (Claim 46) and so as to include the notion that the depletable culture constituents (i.e., the metabolite of Claims 1 and 46) that preclude induction of the inducible promoter by the inducer can be comprised of more than one depletable culture constituent (Claim 50). Claim 53 is drawn to auto-induction of transcription from inducible *lac* promoters in the specific

auto-inducing medias which are fully described in the specification, or combinations of the fully described media. The newly added claims contain no new matter.

In closing, the Applicant submits that the present application is now in condition for allowance and respectfully awaits receipt of a timely Notice of Allowance.

Respectfully Submitted,

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